Validation & Assay Performance Summary

CellSensor® NFκB-*bla* Jurkat Cell Line

Cat. no. K1666

This cell-based assay has been thoroughly tested and validated by Invitrogen and is suitable for immediate use in a screening application. The following information illustrates the high level of assay testing completed and the validation of assay performance under optimized conditions.

Pathway Description

Nuclear Factor Kappa B (NFκB) is a nuclear transcription factor that regulates genes involved in apoptosis, viral defense, cancer, inflammation, and autoimmune disease. TNF alpha binds its receptor, which recruits a protein called TNF receptor death domain (TRADD). TRADD binds TNF receptor associated factor 2 (TRAF-2) which in turn activates NFκB inducible kinase (NIK). NIK phosphorylates proteins that inhibit NFκB in the cytoplasm, thereby marking these inhibitory factors for degradation. NFκB is then able to enter the nucleus and regulate transcription.

Cell Line Description

The CellSensor® NFκB-*bla* Jurkat cell line contains a beta-lactamase reporter gene under control of the Nuclear Factor Kappa Beta (NFκB) response element stably integrated into Jurkat cells. This cell line validated for EC_{50} and Z'-Factor under optimized conditions using Tumor Necrosis Factor Alpha (TNFα). This cell line has also been tested for assay performance under variable experimental conditions, including stimulation time, substrate loading time and DMSO concentration.

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Primary agonist dose response under optimized conditions(n=3)**

TNFα EC_{50}	= 2.4 ng/mL
Z'-Factor (EC_{100})	= 0.88
Response Ratio	= 14
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 0.5%
Recommended Stim.Time	= 5 hours
Max. [Stimulation]	= 0.1 µg/mL

2. **Alternate Stimuli Dose Response**
   See Alternate Stimuli Response Sections

3. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

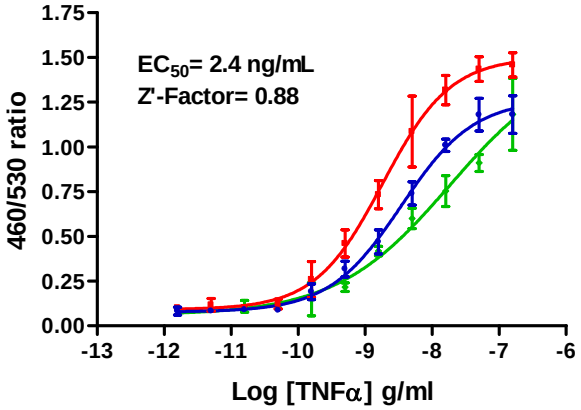
4. **Assay performance with variable stimulation time**

5. **Assay performance with variable substrate loading time**

6. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

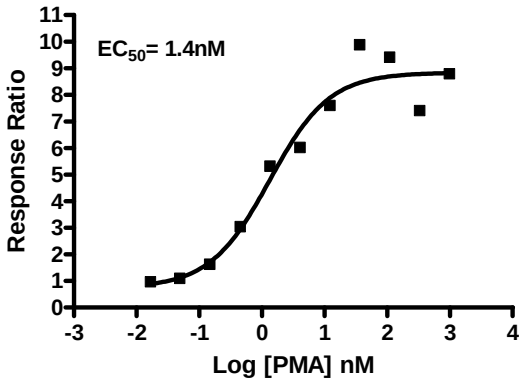
Figure 1 — NFκB-*bla* Jurkat dose response to Tumor Necrosis Factor alpha (TNFα) under optimized conditions



NFκB-*bla* Jurkat cells (20,000 cells/well) were assayed on three separate days represented by the three curves shown on the graph. Cells were plated the day of the assay in a 384-well format and stimulated with TNFα (BD Biosciences # 350466) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Emission Ratios plotted for the indicated concentrations of TNFα (n=16 for each data point).

Alternate Stimuli Response

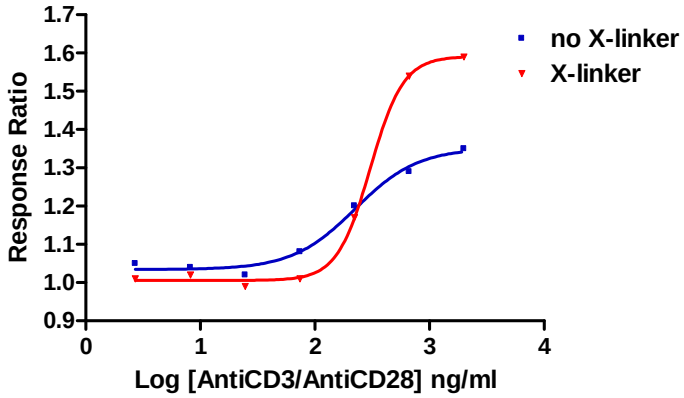
Figure 2 — NFκB-*bla* Jurkat dose response to PMA



NFκB-*bla* Jurkat cells (100,000 cells/well) were plated the day of the assay in a 96-well format and stimulated with Phorbol 12-myristate 13-acetate (PMA); (Sigma# P8139) over the indicated concentration range in the presence of 0.1% DMSO for 6 hours. LiveBLAzer™-FRET B/G substrate was then added to the plate and incubated for 1.5 hrs. Fluorescence Emission values were obtained using a standard fluorescence plate reader and the Response Ratios plotted for the indicated concentrations of PMA.

Alternate Stimuli Response, Continued

Figure 4 — NFκB-*bla* Jurkat cells dose response to Anti CD3/AntiCD28



NFκB-*bla* Jurkat cells (100,000 cells/well) were plated in a 96-well format. Cells were treated with AntiCD3/AntiCD28 (BD Pharmingen #555329 & #555725) with and without goat anti-mouse cross-linker (Pierce #31160) for 5 hrs. Cells were then loaded with LiveBLAzer™- FRET B/G Substrate for 1.5 hrs. Fluorescence emission values at 460 and 530 nm were obtained and the Response Ratios plotted against the indicated concentrations of AntiCD3/AntiCD28.

Cell Culture and Maintenance

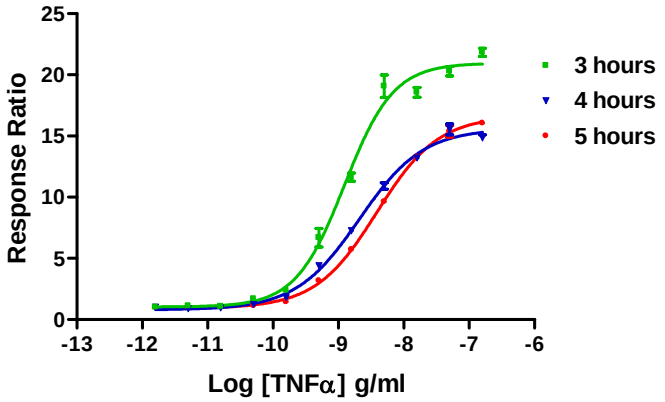
Note: We recommend passing cells for three passages after thawing before using them in the beta-lactamase assay. For proper cell line performance, use dialyzed FBS (Invitrogen# 26400-036). For more detailed cell growth and maintenance directions, please refer to the protocol.

Table 1 – Cell Culture and Maintenance

Component	Growth (-)/ Assay Medium	Growth (+) Medium	Freezing Medium
RPMI 1640	90%	90%	80%
Dialyzed FBS **Do Not Substitute!**	10%	10%	10%
NEAA	0.1 mM	0.1 mM	0.1 mM
Sodium pyruvate	1 mM	1 mM	1 mM
Penicillin (antibiotic)	100 U/ml	100 U/ml	--
Streptomycin (antibiotic)	100 µg/ml	100 µg/ml	--
Blasticidin (antibiotic)	--	5 µg/ml	--
DMSO	--	--	10%

Assay Performance with Variable Stimulation Time

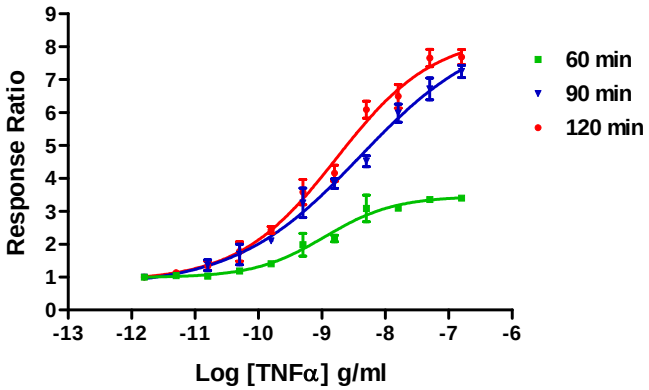
Figure 6 – NFκB-*bla* Jurkat dose response to TNFα with 3, 4 and 5 hour stimulation times



NFκB-*bla* Jurkat cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. TNFα (BD Biosciences # 350466) was then added to the plate over the indicated concentration range. Plates were treated for 3, 4 or 5 hrs with TNFα in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each stimulation time against the indicated concentrations of TNFα (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

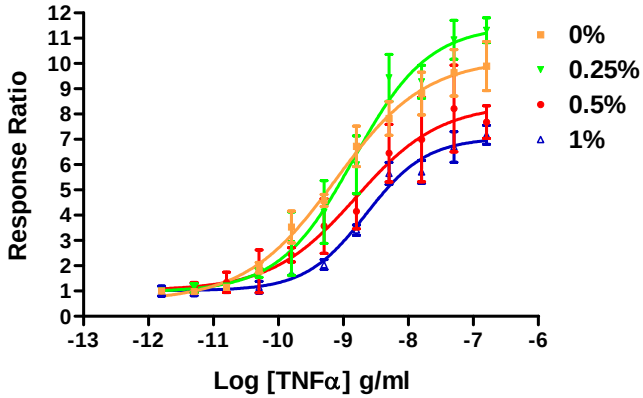
Figure 7 — NFκB-*bla* Jurkat dose response to TNFα with 1, 1.5 and 2 hour substrate loading times



NFκB-*bla* Jurkat cells were plated the day of the assay at 20,000 cells/well in a 384-well format. Cells were treated with TNFα (BD Biosciences # 350466) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5 or 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of TNFα (n=8 for each data point).

Assay Performance with Variable [DMSO]

Figure 6 – NFκB-*bla* Jurkat dose response to TNFα with 0, 0.25, 0.5 and 1% DMSO



NFκB-*bla* Jurkat cells (20,000 cells/well) were plated the day of the assay in a 384-well assay plate. TNFα (BD Biosciences # 350466) was then added to the plate over the indicated concentration range with 0, 0.25, 0.5 or 1% final DMSO concentrations. Cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios for each DMSO concentration were plotted against the indicated concentrations of TNFα (n=8 for each data point).